(3) *Virus titer requirements.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (c)(2) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that when tested at any time within the expiration period, each serial and subserial shall have a virus titer of $10^{0.7}$ times greater than that used in such immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID_{50}'s) per dose.

[44 FR 60263, Oct. 19, 1979, as amended at 44 FR 67087, Nov. 23, 1979; 48 FR 33473, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999; 72 FR 72564, Dec. 21, 2007]

§ 113.332 Tenosynovitis Vaccine.

Tenosynovitis Vaccine shall be prepared from virus-bearing cell culture fluids or embryonated chicken eggs.

Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing seeds for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300, except (a)(3)(ii) and (c), and the special requirements in this section.

(b) Each lot of Master Seed shall be tested for:

(1) Pathogens by the chicken inoculation test prescribed in § 113.36.

(2) Lymphoid leukosis virus contamination as follows:

(i) Each of at least 10 3-week-old or older lymphoid leukosis free chickens from the same source and hatch shall be injected intra-muscularly with an amount of Master Seed equal to 100 label doses of vaccine. At least 15 chickens of the same source and hatch shall be used as controls; 5 or more shall be unvaccinated and serve as negative controls; 5 or more shall be injected with subgroup A lymphoid leukosis virus; and 5 or more with subgroup B lymphoid leukosis virus. Each group of control chickens shall be held isolated from each other and from the vaccinates.

(ii) Twenty-one to 28 days postinoculation, blood samples shall be taken from each chicken and the serum separated using a technique conducive to virus preservation. These serums shall be used as inocula in the complement fixation for avian lymphoid leukosis (COFAL) test prescribed in § 113.31.

(iii) Serums from the vaccinates shall be tested separately, but serums within each control group may be pooled. A valid test shall have positive COFAL reactions from each virus inoculated group and negative reactions from the uninoculated controls. If any of the chickens injected with the Master Seed have positive COFAL test reactions in a valid test, the Master Seed is unsatisfactory.

(3) Identity using the following agar gel immunodiffusion test. The undiluted Master Seed may be used as test antigen or the Master Seed may be inoculated onto the chorioallantoic membrane (CAM) of fully susceptible chicken embryos and the infected CAMs ground and used as antigen. A known tenosynovitis antiserum and a known tenosynovitis antigen shall be used in the test. A precipitin line shall form between the test antigen and the known antiserum in the center well which shows identity with the line formed between the antiserum and the known antigen, or the Master Seed is unsatisfactory.

(4) Safety using the following chicken test:

(i) For vaccines intended for use in chickens less than 14 days of age, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 1-day-old tenosynovitis susceptible chickens.

(ii) For vaccines intended for use only in chickens 14 days of age or older, Master Seed equal to 10 label doses shall be administered subcutaneously to each of 25 4-week-old or older tenosynovitis susceptible chickens.

(iii) The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to

the vaccine, the Master Seed is unsatisfactory. If unfavorable reactions occur which are not attributable to the vaccine, the test is a No Test and may be repeated.

(c) Each lot of Master Seed shall be tested for immunogenicity. The selected virus dose shall be established as follows:

(1) Tenosynovitis susceptible chickens, of the same age and from the same source shall be used as test birds. Vaccines intended for use in very young chickens shall be administered to chickens of the youngest age for which the vaccine is recommended. Vaccines intended for use in older chickens shall be administered to 4-week-old or older chickens. Twenty or more vaccinates shall be used for each method of administration recommended on the label. Ten or more chickens shall be held as unvaccinated controls.

(2) A geometric mean titer of the vaccine produced at the highest passage from the Master Seed shall be established using a method acceptable to Animal and Plant Health Inspection Service before the immunogenicity test is conducted. A predetermined quantity of vaccine virus shall be administered to each vaccinate. Five replicate virus titrations shall be conducted on an aliquot of the vaccine virus to confirm the dose.

(3) Twenty-one to 28 days postvaccination, each vaccinate and control shall be challenged by injecting virulent virus furnished or approved by Animal and Plant Health Inspection Service into one foot pad. The vaccinates and controls shall be observed each day for 14 days. If at least 90 percent of the controls do not develop swelling and discoloration in the phalangeal joint area of the injected foot pad typical of infection with tenosynovitis virus, the test is a No Test and may be repeated. If at least 19 of 20, 27 of 30, or 36 of 40 vaccinates do not remain free from these signs, disregarding transient swelling which subsides within 5 days postchallenge, the Master Seed is unsatisfactory.

(4) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(d) *Test requirements for release.* Each serial and subserial shall meet the applicable general requirements prescribed in §113.300, except (c), and the requirements in this paragraph.

(1) *Purity.* Final container samples of completed product from each serial shall be tested for pathogens by the chicken inoculation test prescribed in §113.36.

(2) *Safety.* (i) Final container samples of completed product from each serial shall be safety tested as follows:

(A) For vaccines intended for use in very young chickens, each of 25 1-day-old tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

(B) For vaccines intended for use in older chickens, each of 25 4-week-old or older tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

(ii) The vaccinates shall be observed each day for 21 days. If unfavorable reactions occur which are attributable to the product, the serial is unsatisfactory. If unfavorable reactions occur in more than two vaccinates which are not attributable to the product, the test is a No Test and may be repeated. If the test is not repeated, the serial is unsatisfactory.

(3) *Virus titer requirements.* Final container samples of completed product shall be titrated by the method used in paragraph (c)(2) of this section. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of the vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer $10^{0.7}$ times greater than that used in the immunogenicity test, but not less than $10^{2.0}$ titration units (PFU or ID_{50}) per dose.

(4) *Identity.* Bulk or final container samples of completed product from each serial shall be tested for identity as prescribed in paragraph (b)(3) of this

section and shall meet the criteria stated therein.

[50 FR 438, Jan. 4, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 64 FR 43045, Aug. 9, 1999; 72 FR 72564, Dec. 21, 2007]

DIAGNOSTICS AND REAGENTS

§§ 113.400–113.405 [Reserved]

§ 113.406 Tuberculin, Intradermic.

Tuberculin, Intradermic, is a filtrate produced from cultures of Pn, C, and Dt strains of *Mycobacterium tuberculosis* (supplied by Animal and Plant Health Inspection Service) which has been inactivated and is non-toxic. Each serial shall be tested for purity, safety, potency, and special chemical tests in accordance with the conditions prescribed for each test. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Each serial shall be tested for purity as provided in this paragraph.

(1) Final container samples of completed product shall be tested for viable bacteria and fungi as prescribed in § 113.26.

(2) A 20 ml sample shall be centrifuged and the sediment examined microscopically for the presence of acidfast (Ziehl-Nielsen stain) or other microorganisms (Gram stain). A serial which contains microorganisms is unsatisfactory for release.

(b) *Safety test.* Final container samples of completed product from each serial shall be tested for safety. Two mature guinea pigs shall be injected subcutaneously with 1 ml and observed for 10 days. If unfavorable reactions attributable to the product occur during the observation period, the serial is unsatisfactory. If unfavorable reactions occur which are not attributable to the product, the test shall be declared a No Test and repeated: *Provided*, That if the test is not repeated, the serial shall be declared unsatisfactory.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be subjected to a comparison test using a Reference Tuberculin supplied by Animal and Plant Health Inspection Service. Test animals shall be 10 sensitized white female guinea pigs from one source which weigh 500–700 grams at the beginning of the test and which have not been used in a previous test. The comparison test shall be conducted in accordance with the procedures prescribed in paragraphs (c)(1), (2), (3), (4), (5), (6), (7), and (8) of this section.

(1) The guinea pigs shall be sensitized with a sterile heat-killed suspension of equal amounts of strains Pn, C, and Dt of *Mycobacterium tuberculosis*. The heat-killed sensitizing agent shall be injected in a volume of 0.5 ml per guinea pig. The guinea pigs shall be considered sensitized for testing not less than 30 days nor more than 120 days post-injection.

(2) The guinea pigs shall be prepared for sensitivity testing at least 4 hours prior to the injection of tuberculin. The entire abdominal and flank areas shall be clipped, a depilatory agent applied for 5–10 minutes, the area rinsed with warm water, and dried.

(3) Dilutions of 1:100, 1:200, and 1:400 shall be prepared with the Reference Tuberculin and the unknown tuberculin. Three test sites on each side of and equidistant from the abdominal midline shall be chosen on each guinea pig. Using a tuberculin syringe and needle, 0.05 ml of each dilution shall be injected intradermally at one of the test sites which has been randomly selected for the dilution.

(4) The sensitivity of the tuberculins shall be determined 24 hours after injected by measuring the area of erythema. Measurements in millimeters shall be made anterior of the greatest diameter and perpendicular to the first measurement. The square millimeter shall be calculated by multiplying the two measurements.

(5) The total area of response for each tuberculin tested shall be determined by adding the areas of erythema for each dilution of each of the test animals in a group. The sums of the areas of erythema for all three dilutions of each tuberculin shall be added to give the total area of tuberculin response.

(6) The total tuberculin response area of the serial being tested shall be expressed as a percentage of the total tuberculin response area of the Reference Tuberculin. (The total response area of the serial divided by the total response